Exhibit 89

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Ovarian Cancer and Asbestos[1]

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There is a rising death rate from ovarian cancer in advanced western countries. By contrast, ovarian cancer is uncommon in less developed areas. Mesotheliomas in asbestosis resemble ovarian cancer in appearance. Intraperitoneal injection of one variety of asbestos produces epithelial changes in the ovaries of guinea pigs and rabbits which are similar to those seen in patients with early ovarian cancer. Six of twelve patients with early ovarian malignant changes show birefringent crystalline material in the ovaries. These observations are compatable with the thesis that asbestos is an etiologic factor in ovarian cancer.

Ovarian cancer is a serious and growing problem. In New York State the death rate of ovarian cancer is 8.4/100,000 females, which is 7% of all female cancer deaths (Handy, 1965). In New York City alone, the death rate from this cause in women 25 years of age or more has increased from 8.7 in 1931 to 20.2 in 1964 (Handy, 1966). This doubling in 33 years is part of a pattern dating from the turn of the century when ovarian cancer was seldom seen. A hundred years ago ovarian cancer was uncommon in both Western Europe and North America. For example: a series from the Royal Infirmary in Edinburgh records the number of cases coming to autopsy for a ten-year period in 1849–1860 (Ogilvie, 1965). There were 15 cancers of the cervix and one cancer of the ovary. In a similar period from 1949–60, there were 23 cervical and 25 ovarian cancers. These two cancers afflict the same age groups. Middlesex Hospital in London from 1855 to 1905 had 1,876 cancers of the cervix, 58 cancers of the uterine body, and a "negligible number" of ovarian cancers (Andreizen and Leitch, 1906). By contrast, "developing" countries now resemble Western Europe 50–100 years ago. For example, in Paraguay in 1960–66, there were 736 histologically confirmed cases of cervical cancer with only 54 ovarian cancers in the same period (Rolon, 1967).

This pattern suggests some element in modern western society that favors the occurrence of ovarian cancer. That element was lacking in the past and its influence has gradually increased. It is apparently still lacking in underdeveloped countries.

CLINICAL STUDIES

Ovarian cancer is usually found in an advanced and relatively incurable stage. In an attempt to detect ovarian cancer earlier, we have applied the same principles of cytologic examination which have proved so successful in discovering early cancer of the cervix. The material used for microscopic examination is peritoneal fluid. The peritoneal fluid is obtained by exposing the posterior vaginal

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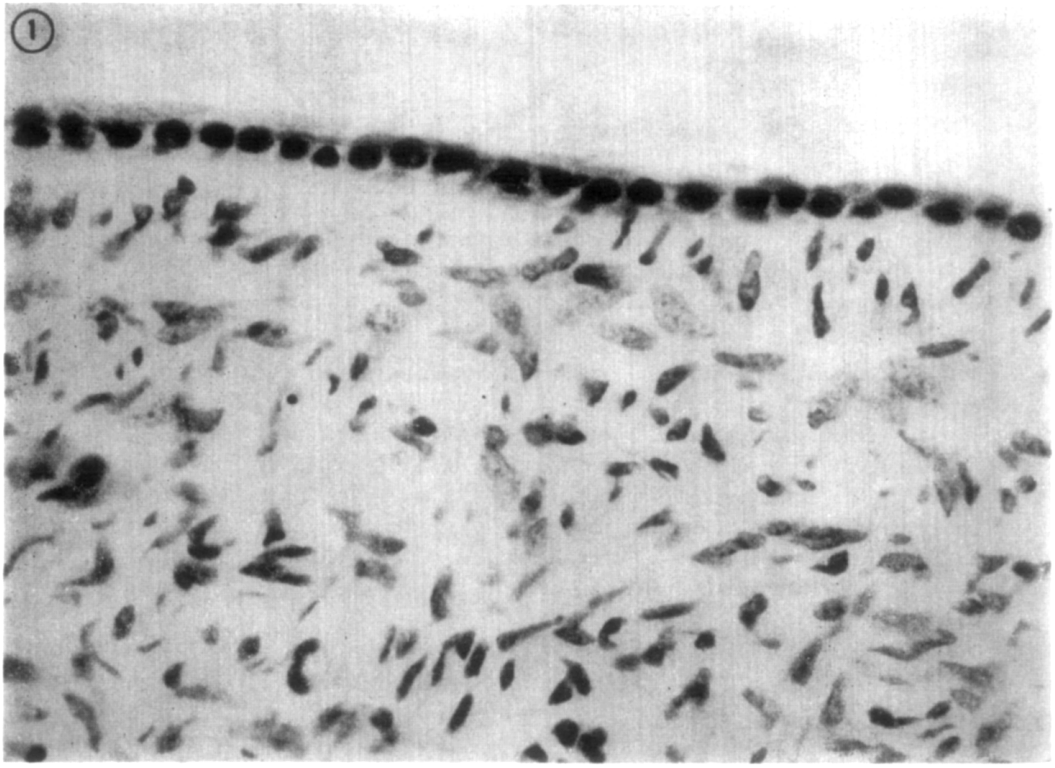

FIG. 1. Cuboidal cells of normal ovarian surface (human).

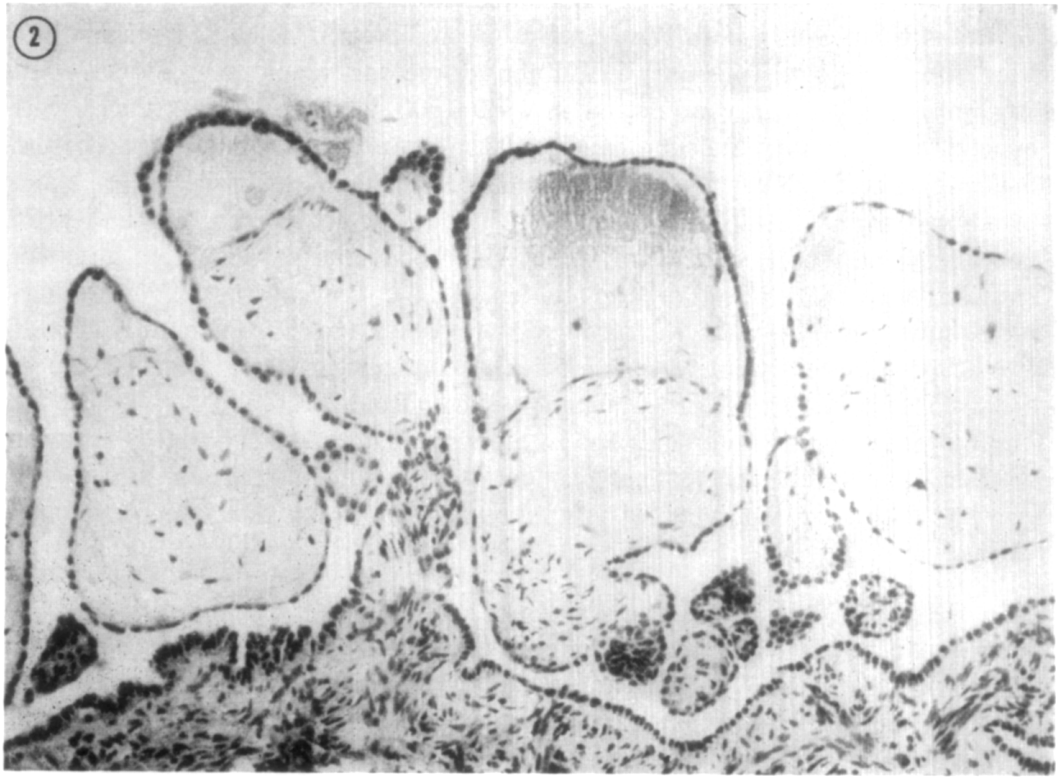

FIG. 2. Extensive papillary processes projecting from ovarian surface (human), with associated positive cul-de-sac fluid. (See Fig. 4.)

fornix with a speculum, passing a needle into the pouch of Douglas, and aspirating fluid. A group of preclinical cases were found. We have reported a series of 22 cases with negative physical findings and positive cul-de-sac aspirations (Graham and Graham, 1967). At surgical exploration, the ovaries were grossly normal in size and appearance. Of these, 20 had borderline lesions and two microinvasive cancer of the ovary.

The histologic patterns found in this group are all indicative of increased mitotic activity. The surface epithelium of the ovary is composed of a single layer of cuboidal cells (Fig. 1). If there is increased cell division in this single-layered epithelium, three changes may occur. First, and most common, is the formation of papillary processes. If mitotic activity is increased, more surface area is necessary and these papillary processes appear to fill this requirement. Figure 2 illustrates this surface abnormality. The entire surface seems to be

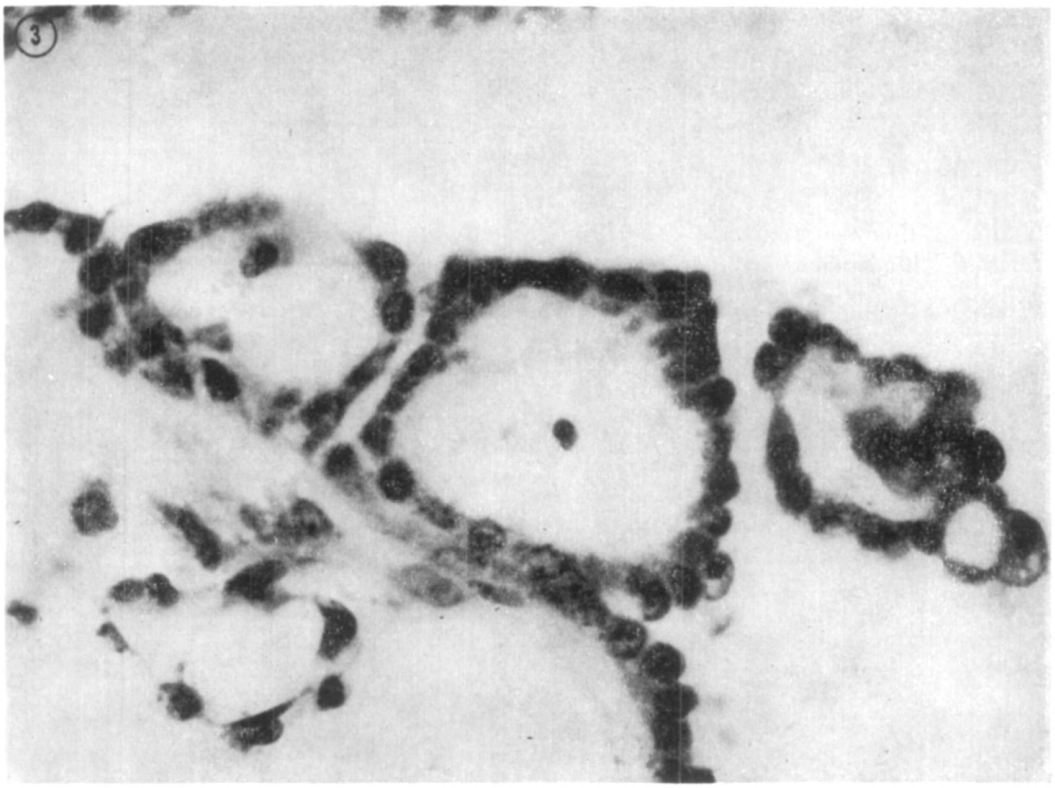

FIG. 3. High-power view of papillary processes—Note irregularity of nuclear material.

bubbling with activity. Higher magnification (Fig. 3) shows that there are marked nuclear abnormalities, especially an irregular chromatin pattern. In the presence of such a markedly abnormal histologic pattern on the surface of the ovary, it is easily understood why malignant cells are found in the aspiration of peritoneal fluid (Fig. 4). The significance of this type of abnormality without stromal invasion is unknown. But that such a lesion may occupy a place in the spectrum of the development of clinical cancer is suggested by the fact that identical lesions are found in the ovaries of patients with clinical cancer (cf. Figs. 5 and 6). Further evidence to substantiate their importance is that the individual cells

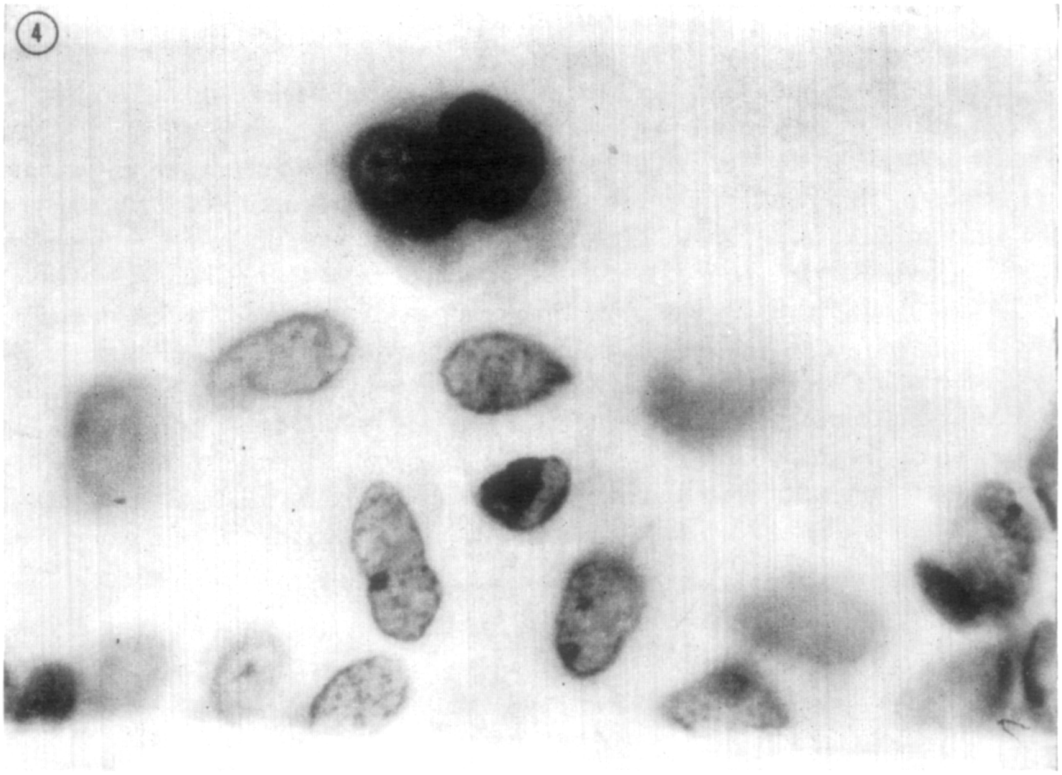

FIG. 4. Cul-de-sac aspiration. The large binucleate cell with hyperchromatic irregular chromatin is malignant. Other cells in the field are normal mesothelial cells. (Same case as Fig. 2.)

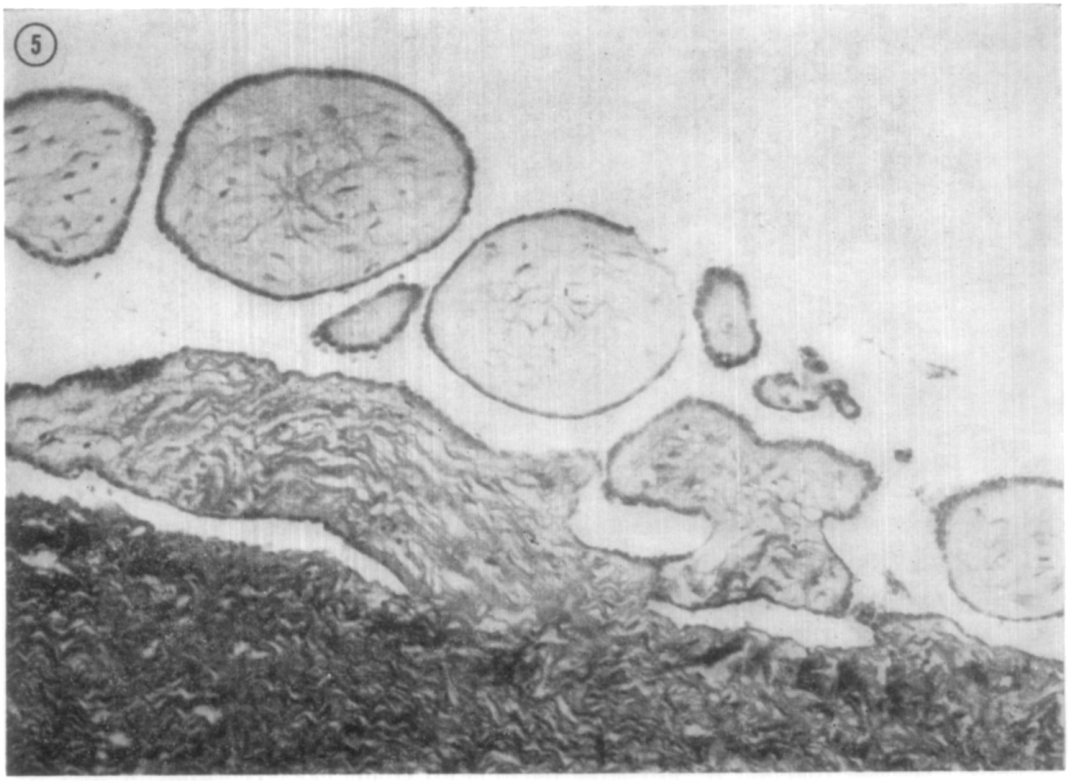

FIG. 5. Papillary processes on ovarian surface in clinical cancer. Other portions of ovary showed invasive carcinoma. Compare with Fig. 2. (human).

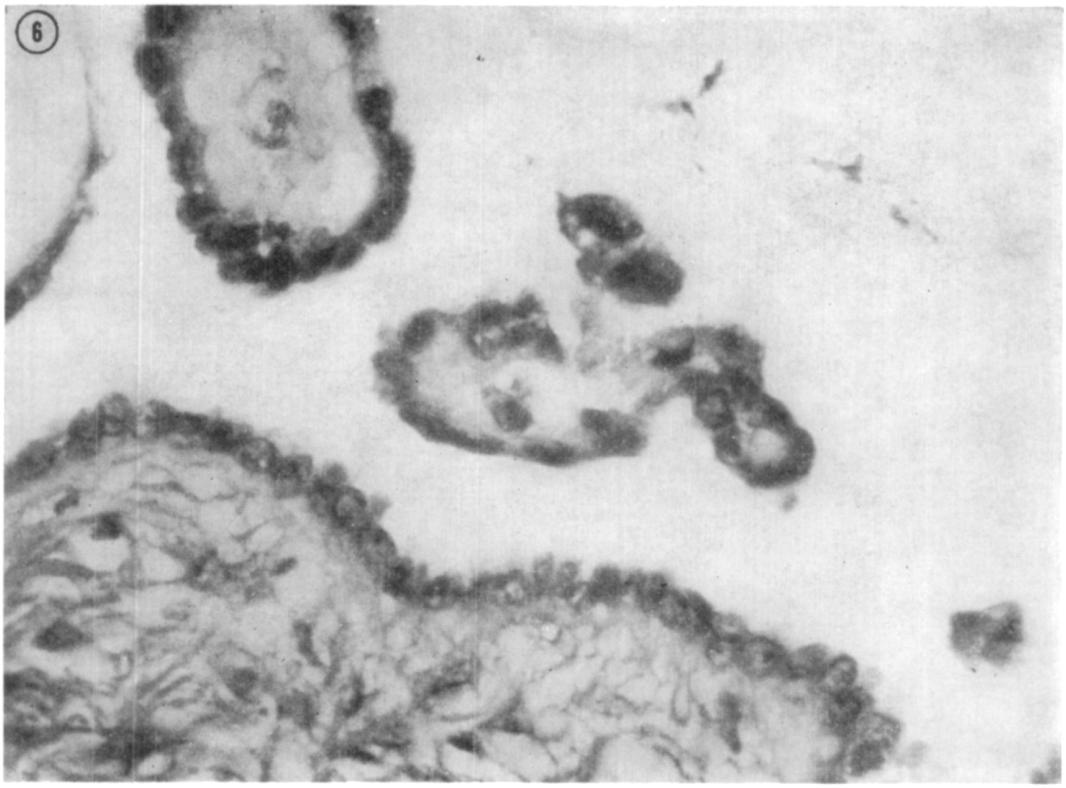

FIG. 6. High-power view of papillary processes shown in Fig. 5. Abnormalities in nuclear structure are present. Compare with Fig. 3.

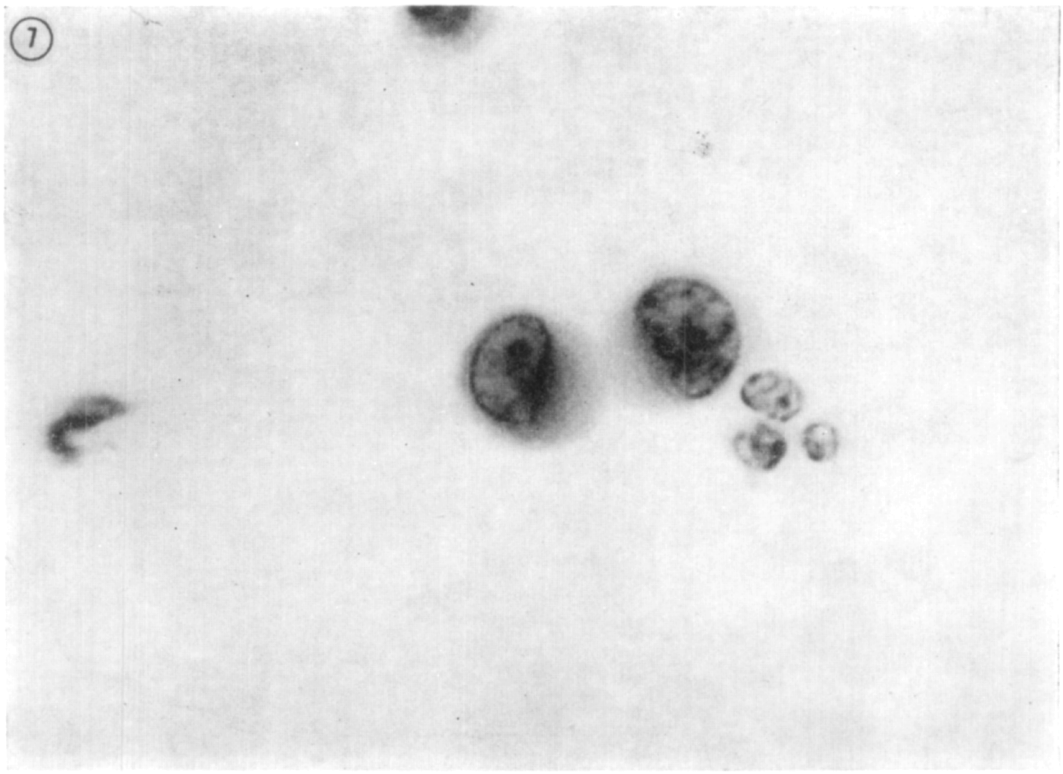

FIG. 7. Two malignant cells found in cul-de-sac aspiration of a patient with clinical carcinoma. Note irregularity of chromatin. Compare with Fig. 4.

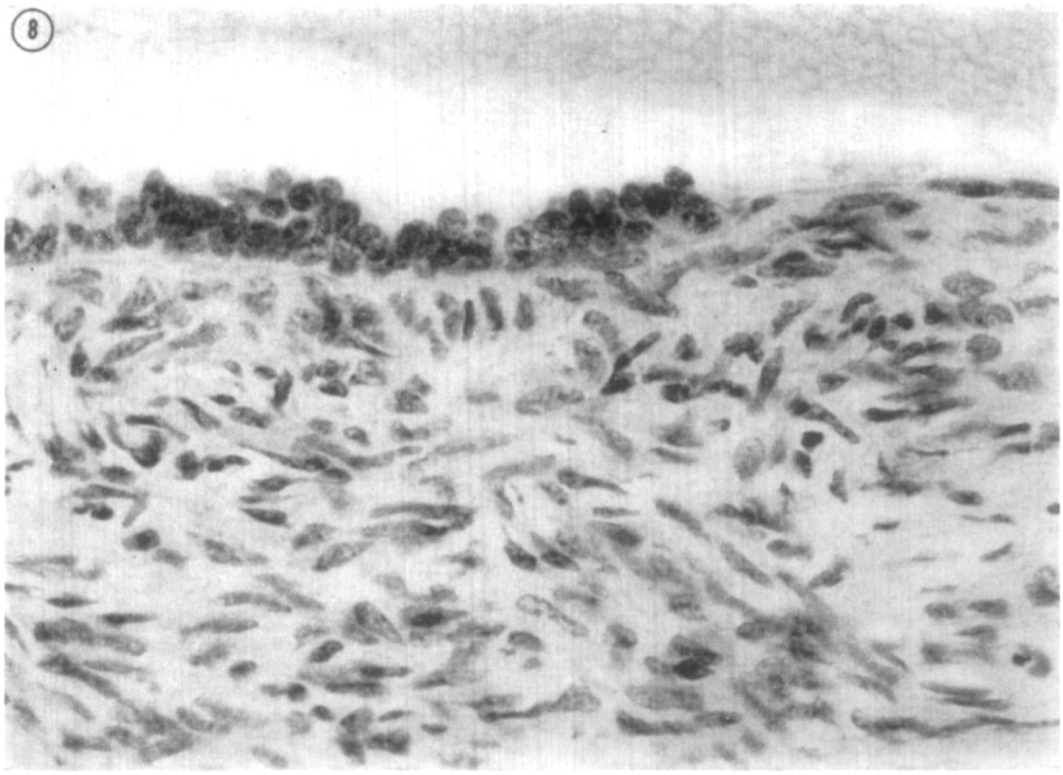

FIG. 8. Multilayering of surface cells, with loss of orientation and nuclear abnormalities (human).

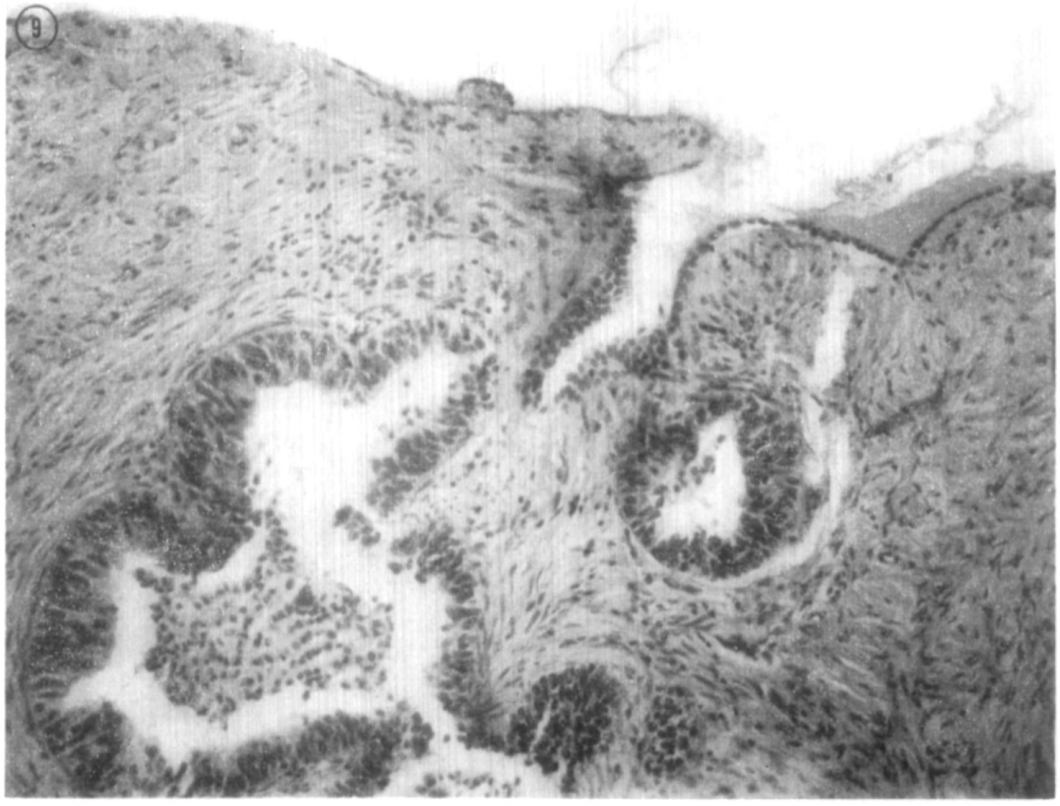

FIG. 9. Invasive carcinoma. Abnormal surface cells have invaded the underlaying stroma (human).

desquamating from such a papillary lesion are indistinguishable from those desquamating from a clinical invasive carcinoma (Fig. 7).

A second change that occurs as the result of increased cellular division is the formation of an abnormal multilayered epithelium (Fig. 8). The individual cells lose their polarity and lie in any direction in relation to the underlying stroma. These multilayered epithelia may be composed of cells with little cytoplasm as illustrated, resulting in poor differentiation. The nuclear material is grossly irregular.

The third change seen in these grossly normal ovaries is invasion of the underlying stroma by proliferation of the surface cells which classifies it as cancer (Fig. 9).

OVARIAN CANCER AND MESOTHELIOMAS

Some mesotheliomas closely resemble ovarian carcinoma histologically. Indeed, in some instances they could not be distinguished one from the other if the anatomical site of origin were not known. To illustrate this, high-power views of an ovarian carcinoma (Fig. 10) and a pleural mesothelioma (Fig. 11) are shown.

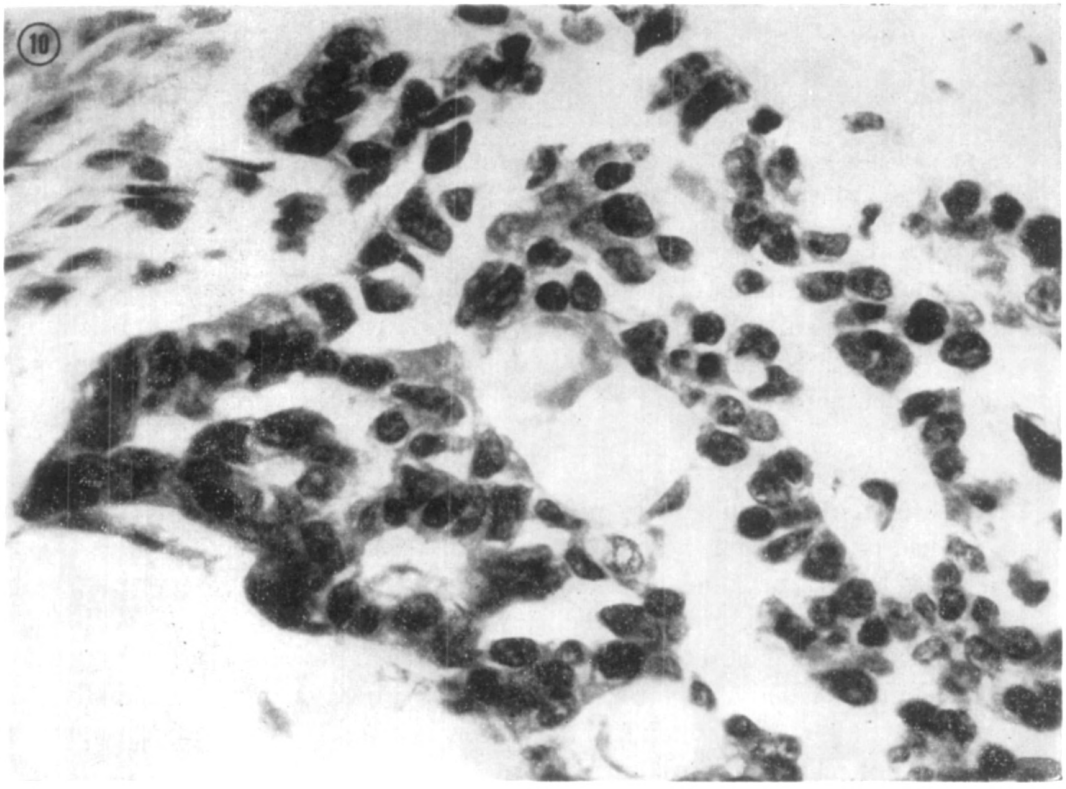

Fig. 10. Omental metastasis from an ovarian carcinoma. Note variation in nuclear structure, irregularly staining cytoplasm and acini (human).

Both tumors are composed of malignant cells showing marked variation in structure with irregularly staining cytoplasm. Both tumors have microscopic acini. Reviewing post-mortem data, Keal (1960) found four lung cancers and nine abdominal cancers in 15 women with asbestosis. One of the abdominal cancers was

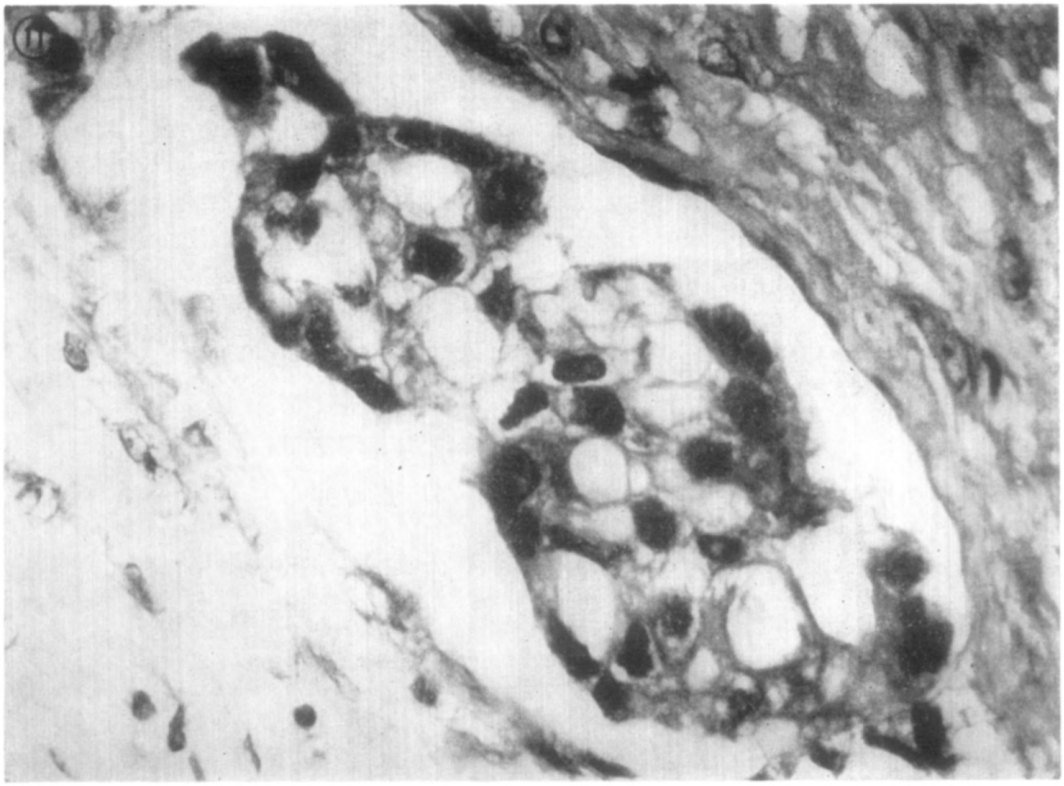

FIG. 11. Pleural mesothelioma. Compare similarity of structure to metastatic ovarian carcinoma, Fig. 10 (human).

definitely ovarian and four were probably ovarian in origin. The remainder were called carcinoma peritonei. This indicates again the difficulty in determining with accuracy whether an abdominal malignacy originated in the surface epithelium of the ovary or in the peritoneum. This is not surprising since one could regard the surface epithelium of the ovary as a specialized mesothelium.

ANIMAL EXPERIMENTS

Because of the morphological similarity of mesotheliomas and ovarian cancer, and because of the strong association of asbestosis with mesotheliomas, we injected asbestos intraperitoneally in four species of animals to observe its possible effect on the ovary. Ten gm of tremolite was stirred for five minutes with 400 cc of tap water in a Waring blender. The mixture was allowed to settle for an hour and the slightly cloudy supernatant fluid was injected intraperitoneally. The injection schedule was as follows: 0.1 cc in Swiss mice, 0.2 cc in Syrian hamsters, 0.5 cc in guinea pigs, 1.0 cc in Dutch rabbits. A single injection was given initially. In week 10, weekly injections were begun and continued until week 18 (the end of the present study). Animals were killed at 1–4 week intervals. The ovaries were fixed in Carnoy's fluid and paraffin sections made.

RESULTS

No abnormalities were found in nineteen hamsters or in eighteen mice. Three noninjected hamsters and three control mice were identical to the injected group. The lack of any change in these two species may be explained by the fact that

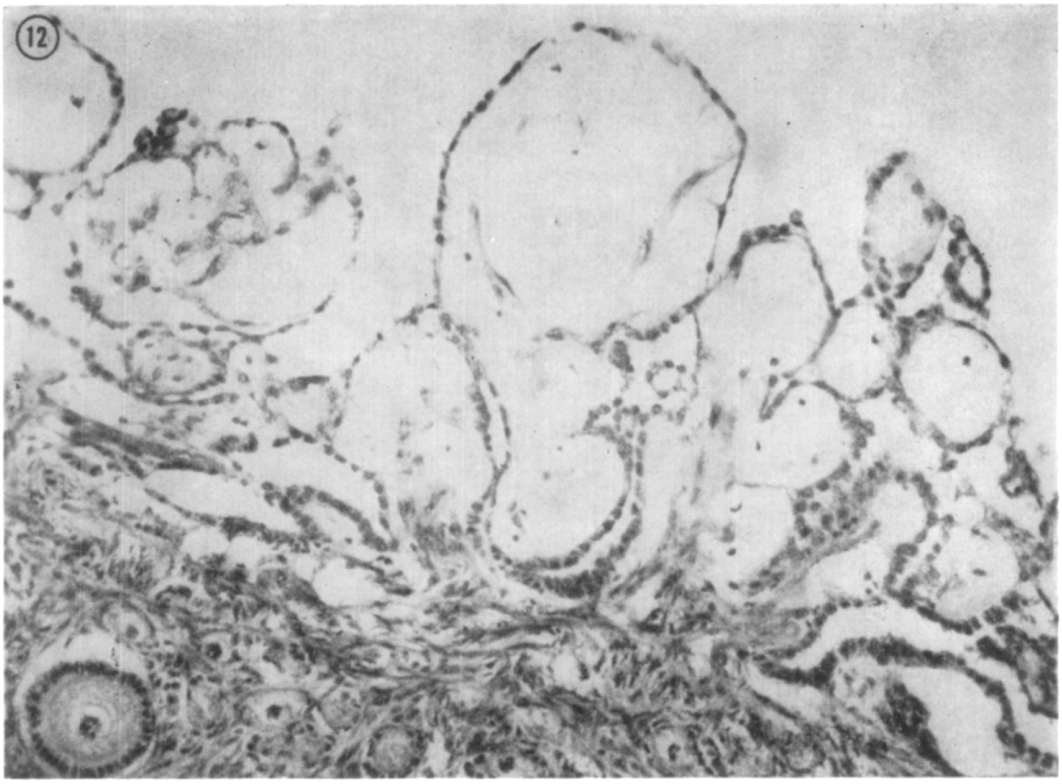

FIG. 12. Marked proliferation of papillary process in rabbit injected intraperitoneally with asbestos seven weeks previously; compare with Figs. 2 and 5.

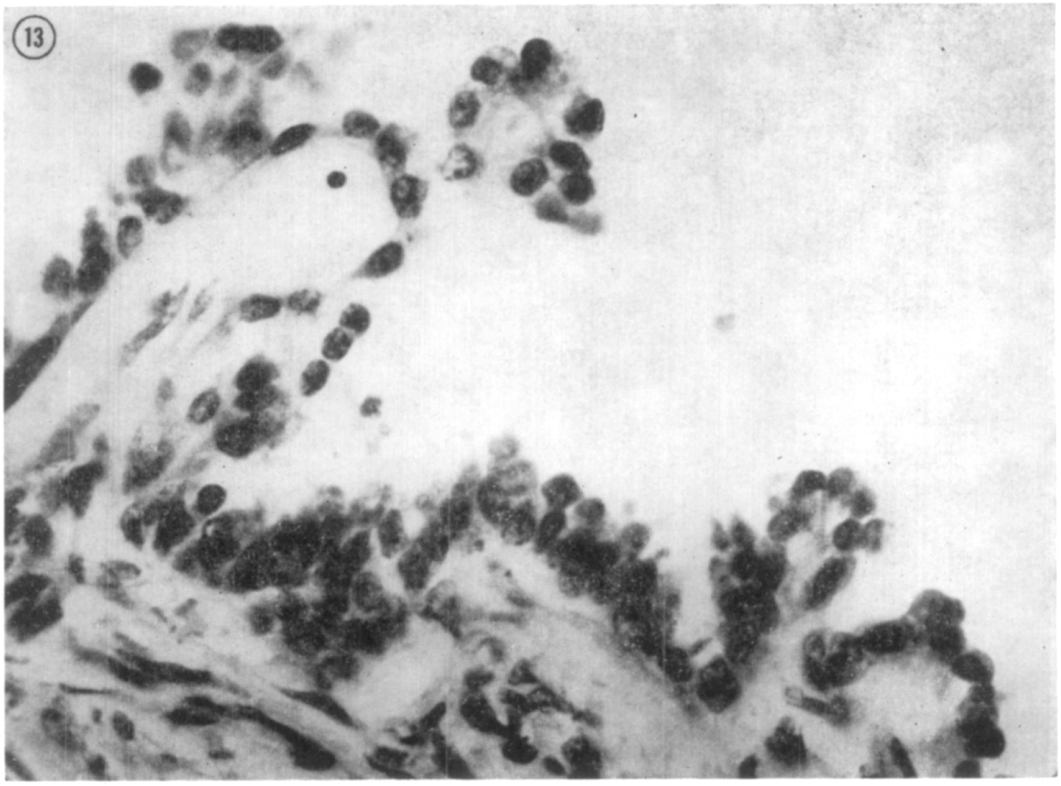

FIG. 13. High-power view of papillary surface projections in the rabbit. Compare with Figs. 3 and 6.

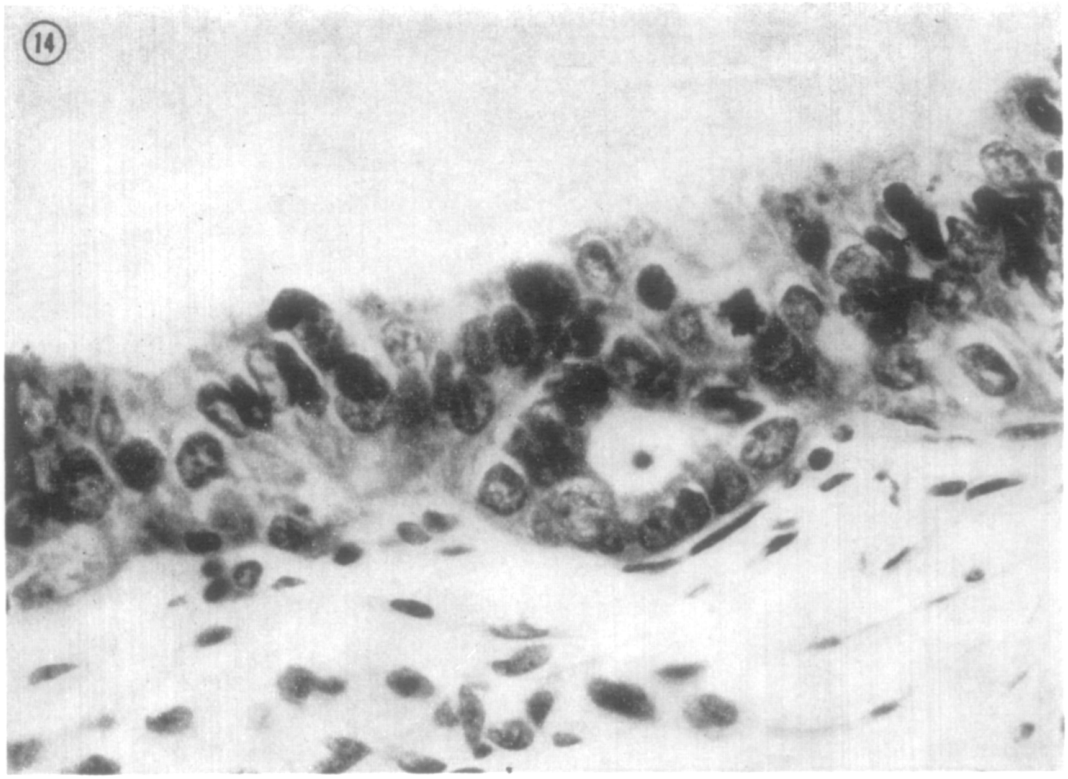

FIG. 14. **Multilayering of surface epithelium in the ovarian surface of a guinea pig injected with asbestos at week 17. Loss of orientation and abnormal nuclei are present. An abnormal mitotic figure can be seen. Compare with Fig. 8.**

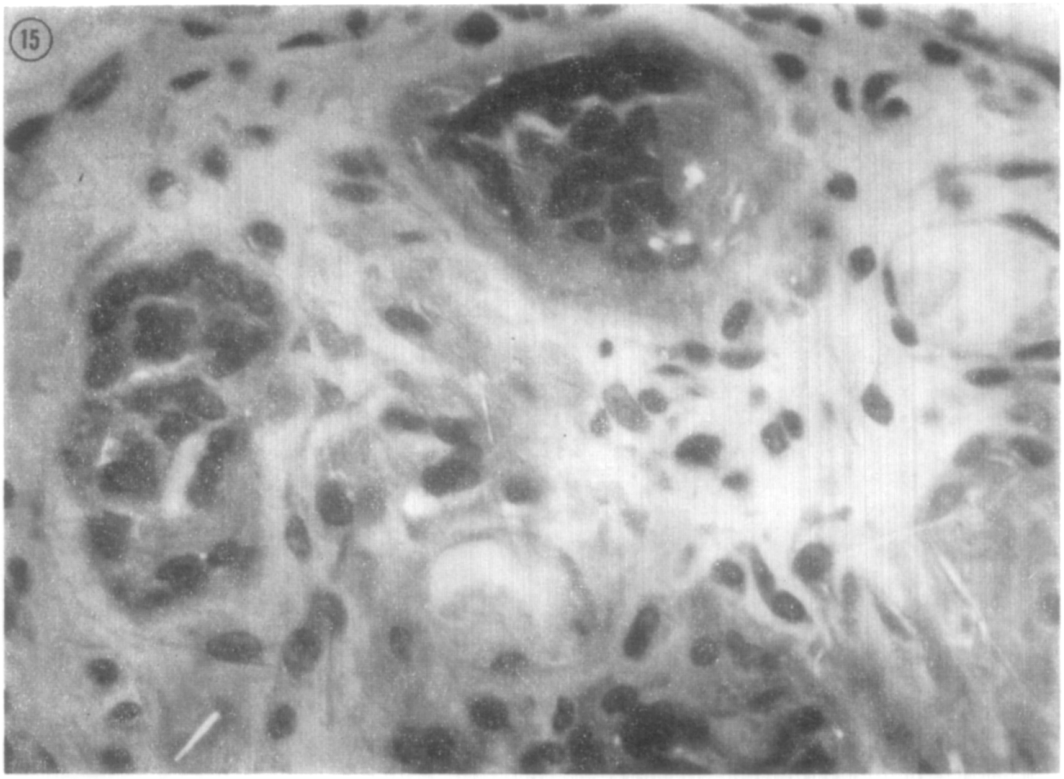

FIG. 15. **Asbestos particles in foreign body giant cells in a granuloma of the peritoneum. Polarized light (rabbit).**

in the mouse and hamster the ovary is surrounded by a veil of peritoneum which make it inaccessible to injected material.

Ten rabbits were injected intraperitoneally and two showed surface abnormalities, one at week 6 and one at week 16. The ovarian surface was converted from a single layer of cuboidal epithelium to an extensive papillary process of cells with abnormal nuclear configuration (Figs. 12 and 13) reminiscent of changes seen in the early ovarian lesions in the human (compare with Fig. 2 and Fig. 3). The ovaries of ten rabbits which had received no injection were examined as controls. None showed any abnormalities.

Sixteen guinea pigs were injected and two showed abnormalities at weeks 7 and 17. The surface changes in the ovaries of the guinea pigs were a transformation of the single layer epithelium (Fig. 14) to a multilayered one with extremely abnormal cells and mitotic activity. This, too, is similar to the lesions seen in the early ovarian lesions in humans (Fig. 8). Sixteen noninjected guinea pigs served as controls. There were no abnormalities in the ovaries.

Tissues of animals injected with asbestos and killed up through week 15 had no visible asbestos fibers. Rabbits killed in week 18 had numerous fibers and much foreign body reaction (Fig. 15) apparently the result of the injection of coarser material.

FOREIGN BODY REACTION IN HUMAN OVARIES

Asbestos fibers can be demonstrated in the lungs of patients with asbestosis, but they are difficult or impossible to show in fully developed mesotheliomas.

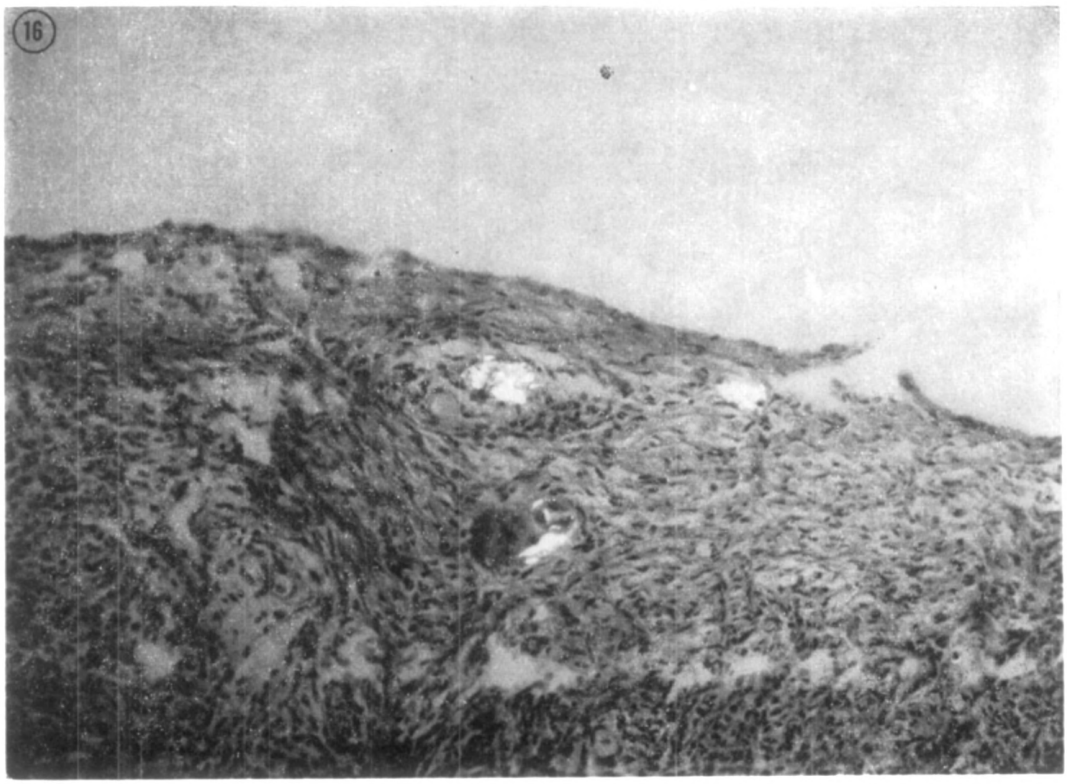

FIG. 16. Low-power view of ovary showing three cluster of birefringent particles—polarized ight (human).

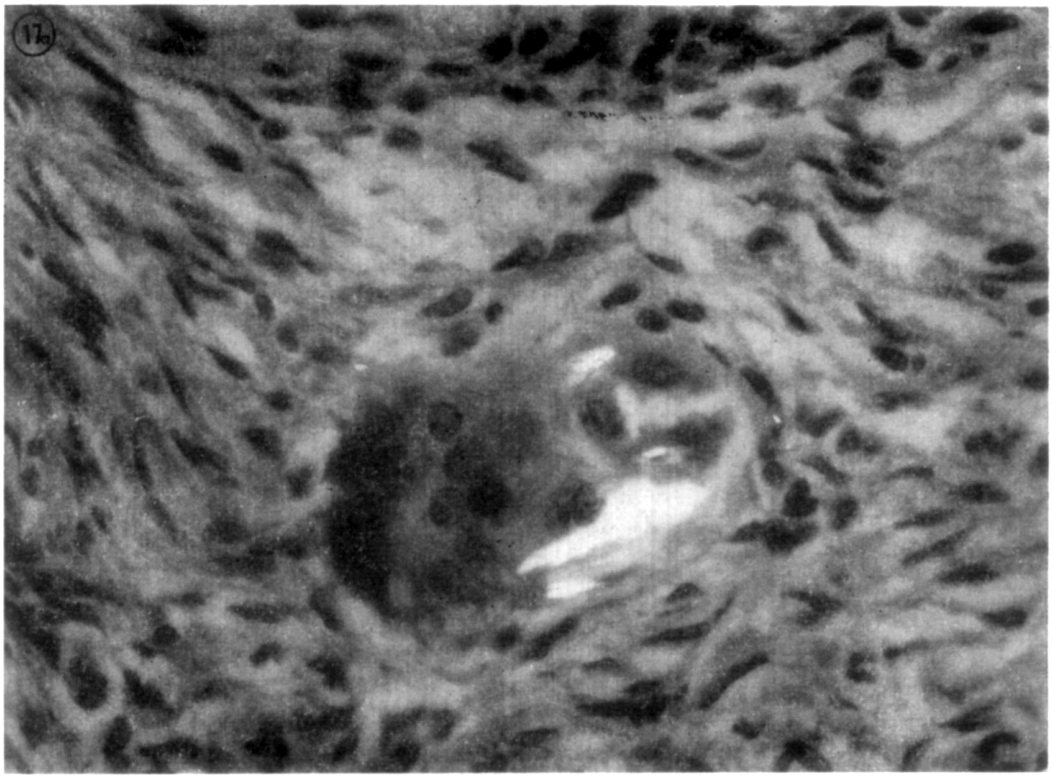

FIG. 17a. High-power view of a foreign body giant cell, seen in Fig. 16, containing birefringent particles of different size polarized light.

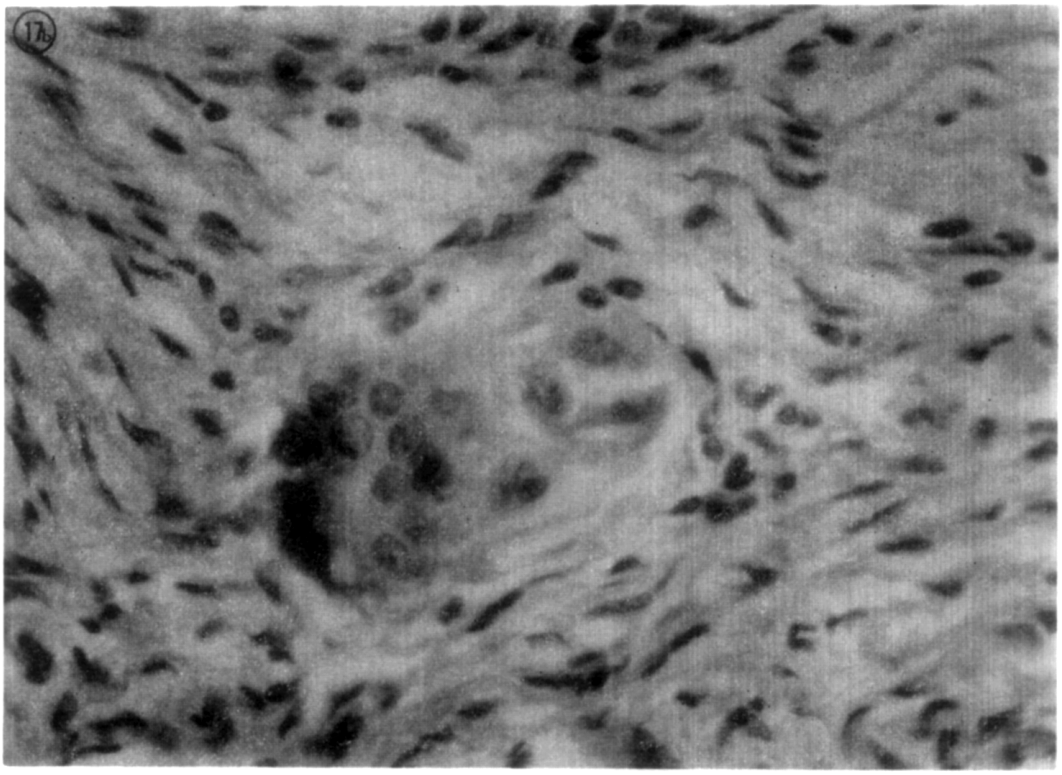

FIG. 17b. Same cell as in Fig. 17a photographed in ordinary light. The particles are less visible.

Special techniques have been developed to demonstrate asbestos fibers, including tissue ashing and electron microscopy. A simple and rather crude method is to use polarized light. Asbestos and other birefringent crystals will appear brightly illuminated.

Stained histologic sections from twelve ovaries showing borderline or microinvasive carcinoma were examined in this way. In six foci were found composed of histiocytes and foreign body giant cells about intact crystals (Figs. 16, 17 a and b). In most instances, these foci were near abnormal surface changes. The crystals have not been identified. Normal ovaries from nine patients showed no crystalline material.

DISCUSSION

Ovarian cancer has been reported in association with asbestosis. Keal (1960) found four lung cancers and nine abdominal cancers in 15 women (with asbestosis) who died. The interval from first exposure to cancer ranged from 21 to 46 years. In 15 men dying with asbestosis, 10 had lung cancer and only one carcinoma peritonei, which suggests a vulnerability of the lung in males and the abdominal area in females.

Adenoid proliferation of the terminal bronchioles in a guinea pig is reported seven months after the beginning of prolonged exposure to asbestos dust (Holt *et al.*, 1966). This perhaps is comparable to the ovarian surface changes observed here.

Asbestos is one of many substances related to modern western civilization. Its production has increased 1,000-fold in the past 60 years which is more than the 50-fold increase in petroleum in the same period (Gilson, 1965). Asbestos is ubiquitous. It is used in housing, electrical appliances, in brake linings and clutches in automobiles, for paving, and in a host of other less conspicuous sites closely related to our everyday life. Asbestos is virtually indestructable. It can be fragmented into the finest dust but not destroyed. There is little doubt that asbestos produces pleural and peritoneal mesotheliomas in asbestos workers (Selikoff *et al.*, 1965). They also suffer a higher than expected death rate from gastrointestional cancer (Selikoff *et al.*, 1964). The time interval of exposure to death is quite long, measured in decades. Asbestos given to animals as inhaled dust or by injection will produce mesotheliomas or lung tumors after many months (Peacock and Peacock, 1965; Smith *et al.*, 1965).

This web of circumstantial evidence associates asbestos with ovarian cancer. There are numerous lacunae. However, the observations are sufficiently suggestive to encourage further work. The possible etiologic relationship demands a fuller account.

ACKNOWLEDGMENT

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